PATENT
Attorney Docket No.: 019941-001810US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No. 5398
Yuuki Tsutsui, *et al.*	Examiner: Bruce D. Hissong
Application No.: 10/674,581	Technology Center/Art Unit: 1646
Filed: September 29, 2003	DECLARATION
For: IMMUNE RESPONSE INDUCTION METHOD	
Customer No.: 20350	

I, Yuuki Tsutsui, being duly warned that willful false statements and the like are punishable by fine or imprisonment or both, under 18 U.S.C. § 1001, and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. I am a citizen of Japan, and I have received the degree of master in pharmacology from Nagoya City University. My *Curriculum Vitae* is attached as Exhibit 1.

2. I have been employed by Astellas Pharma Inc. (formally Yamanouchi Pharmaceutical Co., Ltd., the assignee of the present invention) since 1995.

3. I have been engaged in research and development regarding pharmaceutical preparations since joining Astellas.

4. I am a co-inventor of the inventions described in the specification of the above-identified application (hereinafter, referred to as the "present application").

5. As described herein, a study was undertaken to examine the efficacy of interferon α ("IFNα") as a mucosal adjuvant, which is a technological feature of the present

invention, in comparison against interferon β ("IFNβ"), as well as cholera toxin B subunit (CTB). IFNβ is a cytokine adjuvant and CTB has been reported to be very effective as a mucosal adjuvant. The purpose of the study was to evaluate the characteristics and efficacy of IFNα as a mucosal adjuvant.

6. In comparing IFNα against IFNβ, ovalbumin (OVA) was used as a vaccine antigen. The IFNα or IFNβ at a dose of 4000U along with the OVA was intra-nasally administered to C57BL mice (8-week old) three times (Day 0, 7, and 14). Blood and nasal mucosa were collected on Day 15, 21 (3w), and 28 (4w) after the first administration Day 0, and the levels of OVA-specific antibodies (IgG and IgA) in each sample were measured by ELISA.

7. In comparing IFNα against CTB, ovalbumin (OVA) was used as a vaccine antigen. IFNα at a dose of 4000U or 1 μg of CTB along with the OVA was intra-nasally administered to C57BL mice (8-week old) three times (Day 0, 7, and 14). Blood and nasal mucosa were collected on Day 15, 21 (3w), and 28 (4w), and the levels of OVA-specific antibodies (IgG and IgA) in each sample were measured by ELISA.

8. At a dose of 4000U, IFNβ and IFNα, both were confirmed to induce comparable levels of circulating antigen-specific IgG (Fig. 1). On the other hand, examination of the antigen-specific IgA excreted from the nasal mucosa confirmed that IFNα surprisingly has a higher antibody-inducing ability compared with IFNβ (Fig. 2). These results illustrate that IFNα is a cytokine having an unexpected higher mucosal adjuvant efficacy in comparison with IFNβ.

9. In addition, IFNα was also compared against CTB, which is a very effective mucosal adjuvant. The dose of CTB applied was 1μg, which has been reported in the literature to exert mucosal adjuvant efficacy. The dose of IFNα applied was 4000U. Both were confirmed to induce comparable levels of circulating antigen-specific IgG (Fig. 3). On the other hand, examination of the antigen-specific IgA excreted from the nasal mucosa confirmed that IFNα has a high antibody-inducing ability. The results illustrate that IFNα has a high mucosal adjuvant efficacy.

10. CTB at high doses is widely known to cause adverse effects such as diarrhea, whereas IFNα is applicable at much higher doses in view of known clinical applications. Thus, in application to humans, it is my scientific opinion that IFNα is expected to be useful as a safer and more practical mucosal adjuvant than CTB.

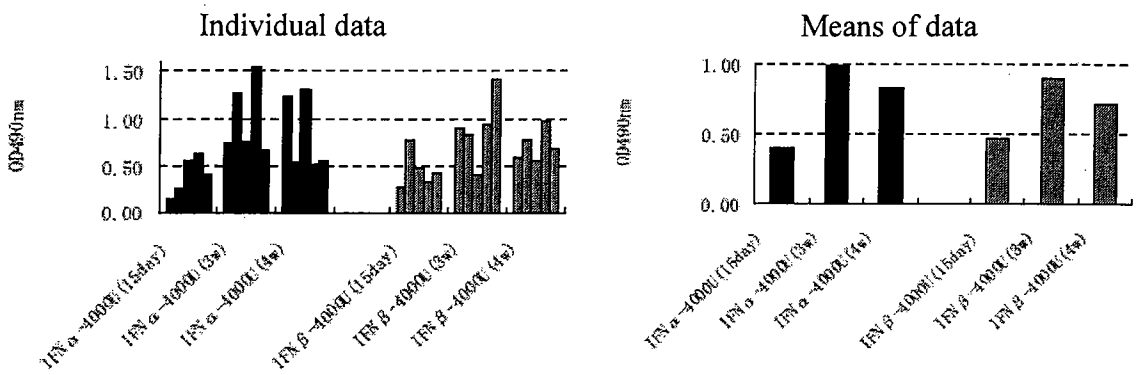

Fig. 1 Results of the comparison between the circulating OVA-specific IgG-inducing abilities of IFNα and IFNβ.

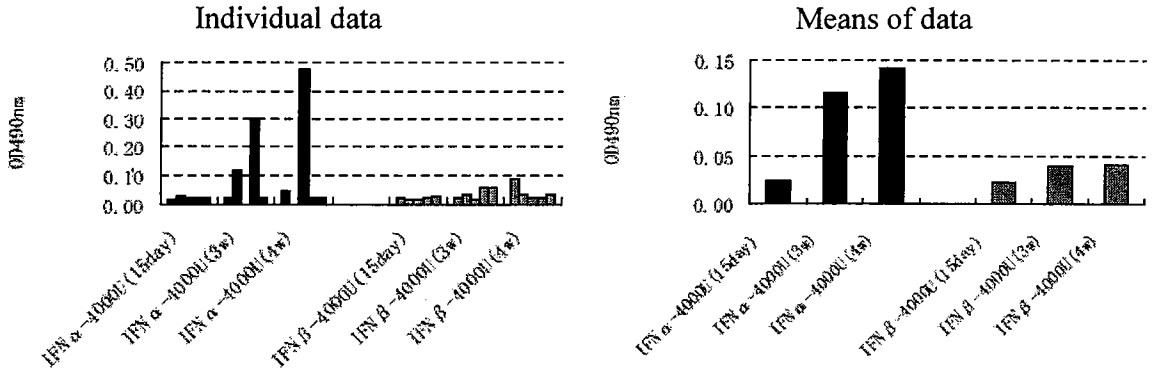

Fig. 2 Results of the comparison between the nasal mucosal OVA-specific IgA-inducing abilities of IFNα and IFNβ.

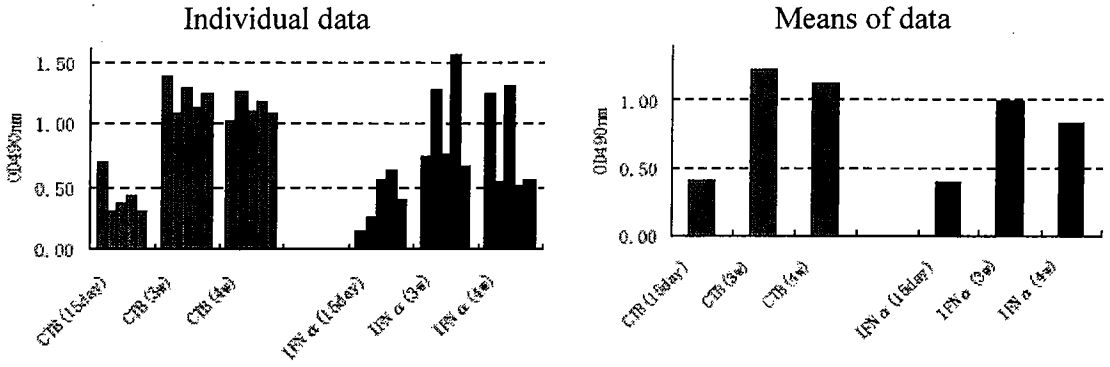

Fig. 3 Results of the comparison between the circulating OVA-specific IgG-inducing abilities of IFNα and CTB.

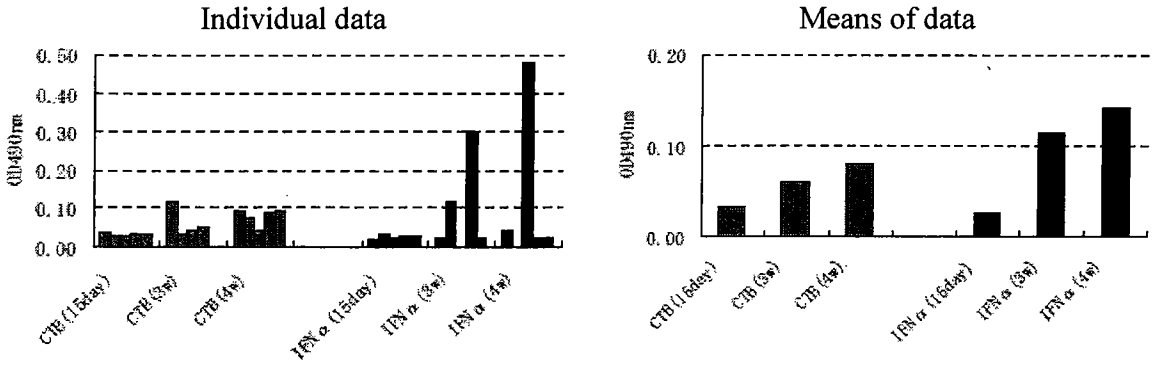

Fig. 4 Results of the comparison between the nasal mucosal OVA-specific IgA-inducing abilities of IFNα and CTB.

The declarant has further nothing to say.

Yuuki Tsutsui

Yuuki Tsutsui

2008. 6. 24

Date

61344654 v1

Exhibit 1

Yuuki Tsutsui's *Curriculum Vitae*:

I have received the degree of Faculty of Pharmaceutical Science in March 1993 from Nagoya City University and the degree of Master in Pharmacology in March 1995 from Nagoya City University.